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2013. Genome-Wide Effects of Selenium and Translational Uncoupling on Transcription in the Termite Gut Symbiont *Treponema primitia*. mBio 4(6): .
doi:10.1128/mBio.00869-13.

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RESEARCH ARTICLE

Genome-Wide Effects of Selenium and Translational Uncoupling on Transcription in the Termite Gut Symbiont *Treponema primitia*

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ABSTRACT When prokaryotic cells acquire mutations, encounter translation-inhibiting substances, or experience adverse environmental conditions that limit their ability to synthesize proteins, transcription can become uncoupled from translation. Such uncoupling is known to suppress transcription of protein-encoding genes in bacteria. Here we show that the trace element selenium controls transcription of the gene for the selenocysteine-utilizing enzyme formate dehydrogenase (*fdhF_{Sec}*) through a translation-coupled mechanism in the termite gut symbiont *Treponema primitia*, a member of the bacterial phylum *Spirochaetes*. We also evaluated changes in genome-wide transcriptional patterns caused by selenium limitation and by generally uncoupling translation from transcription via antibiotic-mediated inhibition of protein synthesis. We observed that inhibiting protein synthesis in *T. primitia* influences transcriptional patterns in unexpected ways. In addition to suppressing transcription of certain genes, the expected consequence of inhibiting protein synthesis, we found numerous examples in which transcription of genes and operons is truncated far downstream from putative promoters, is unchanged, or is even stimulated overall. These results indicate that gene regulation in bacteria allows for specific post-initiation transcriptional responses during periods of limited protein synthesis, which may depend both on translational coupling and on unclassified intrinsic elements of protein-encoding genes.

IMPORTANCE A large body of literature demonstrates that the coupling of transcription and translation is a general and essential method by which bacteria regulate gene expression levels. However, the potential role of noncanonical amino acids in regulating transcriptional output via translational control remains, for the most part, undefined. Furthermore, the genome-wide transcriptional state in response to translational decoupling is not well quantified. The results presented here suggest that the noncanonical amino acid selenocysteine is able to tune transcription of an important metabolic gene via translational coupling. Furthermore, a genome-wide analysis reveals that transcriptional decoupling produces a wide-ranging effect and that this effect is not uniform. These results exemplify how growth conditions that impact translational processivity can rapidly feed back on transcriptional productivity of prespecified groups of genes, providing bacteria with an efficient response to environmental changes.

Received 10 October 2013 Accepted 15 October 2013 Published 12 November 2013

Citation Matson EG, Rosenthal AZ, Zhang X, Leadbetter JR. 2013. Genome-wide effects of selenium and translational uncoupling on transcription in the termite gut symbiont *Treponema primitia*. mBio 4(6):e00869-13. doi:10.1128/mBio.00869-13.

Editor Margaret McFall-Ngai, University of Wisconsin

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The direct coupling of transcription and translation is a characteristic unique to prokaryotes because both processes occur simultaneously in the same compartment. The coupling and uncoupling of these processes are responsible for the well-characterized regulatory mechanism known as attenuation, exemplified by the tryptophan biosynthesis (*trp*) operon of *Escherichia coli* (1). In this mechanism of gene regulation, common in several amino acid biosynthesis pathways and in many bacteria, alternative stem-loop mRNA secondary structures can be formed or resolved as a result of stalled or actively translating ribosomes. These alternative RNA structures regulate the transcription of downstream genes by inhibiting or allowing processive transcription by RNA polymerase (RNAP) (1). The relationship between transcription and translation also gives rise to polar effects in bacteria, first observed by Zipser in 1969 (2). In this phenomenon, point mutations and reading frame shifts that introduce translational stop codons in protein-coding genes signal

the end of translation and thus prematurely uncouple translation from transcription, inhibiting transcription of genes downstream of the site of an introduced lesion within an operon.

Interactions between translational machinery and transcriptional machinery can also govern the rate of transcription of bacterial genes. In bacterial cells, RNAP functions via a Brownian ratchet mechanism wherein forward progression and backtracking both occur in an ATP-independent manner (3). In highly transcribed but nontranslated genes, such as rRNAs, multiple RNAPs operating in succession mechanically limit backtracking and contribute to high transcriptional throughput (4). The capacity of ribosomes to influence transcriptional rates has also been documented. The mechanics of ribosome and RNAP interactions can involve direct contact or indirect contact through other protein factors, including NusG and NusE (5) and the transcriptional termination factor Rho. Proshkin and colleagues (6) have demonstrated the finely tuned coupling of translation and transcription by comparing genes containing frequently

used codons with genes containing infrequently used codons, which are translated at a lower rate and are consequently transcribed at a lower rate, matching that of translation. The authors thus concluded that macromolecular interactions between translational and transcriptional machinery are the fundamental mechanism of post-initiation gene regulation and adaptation to environmental changes in bacteria (6).

The synthesis of some proteins inevitably depends on the ability of the translational machinery to decode extremely rare codons. This is the case for proteins that utilize the noncanonical, twenty-first amino acid, selenocysteine, found in the catalytic active site of a variety of enzymes. Selenium substitutes for the sulfur moiety in the thiol R group of cysteine in the formation of selenocysteine (7). The incorporation of selenocysteine into proteins requires dedicated cellular machinery because it is encoded by UGA, a codon normally used to signal a translational stop. A specialized translation elongation factor (SelB) recognizes a stem-loop mRNA structure known as a selenocysteine incorporation sequence (SECIS) element located downstream from selenocysteine codons and directs ribosomes to incorporate selenocysteine into the nascent polypeptide chain (8, 9). A lack of selenium (and thus selenocysteine) should prevent ribosomes from correctly translating UGA codons as selenocysteine and instead pause or terminate translation.

The homoacetogenic spirochete *Treponema primitia* has two genes that encode formate dehydrogenase isoenzymes, allowing this organism to grow lithotrophically on H_2 plus CO_2 (10, 11). One version of the gene (*fdhF_{Cys}*) encodes an enzyme that utilizes cysteine in the active site, while the other (*fdhF_{Sec}*) encodes an enzyme that uses the noncanonical amino acid selenocysteine. Studies have shown that selenocysteine-containing enzymes can be orders of magnitude more active than cysteine-containing homologs (12–16). Consistent with the hypothesis that the selenocysteine-containing enzyme is preferred in *T. primitia* because it may be more active, we previously showed that transcription of the selenocysteine version of the gene is favored over the cysteine version when a source of selenium is available. Although selenium levels influenced the transcription of *fdhF_{Cys}* and *fdhF_{Sec}*, it appeared to specifically prevent full-length transcription of *fdhF_{Sec}*. The transcriptional pattern of *fdhF_{Sec}* suggested that selenium regulation did not affect transcriptional initiation but instead influenced transcriptional elongation downstream from the promoter such that production of the full-length transcript is dependent on the presence of the trace element in the medium. A report by Liu et al. (17) in which the authors synthetically engineered an attenuation mechanism based on the ability of bacterial cells to incorporate a rare (unnatural) amino acid provides an elegant example of how a transcription/translation coupling mechanism might function within the open reading frame of a gene, but such a system has not been reported to occur in nature. In this study, we demonstrate that the naturally occurring selenium regulation of *fdhF_{Sec}* transcription proceeds via a similar mechanism of uncoupling of transcription from translation, which explains our previous observation of the transcriptional patterns of this gene.

To examine the influence of translation on transcription of protein-encoding genes in *T. primitia*, we included the first application, to our knowledge, of high-throughput sequencing to precisely track genome-wide transcriptional effects of uncoupling translation from transcription. Our results demonstrate that the

availability of the trace element selenium influences transcription of *fdhF_{Sec}* in *T. primitia* through a translational-coupled mechanism involving the incorporation of the noncanonical selenocysteine amino acid and provide additional details on the effect. Moreover, our genome-wide transcriptional data show that while translation does indeed broadly influence transcription of functional genes, consistent with the results of Proshkin et al. (6), translation alone does not necessarily or uniformly govern transcription. Specific post-initiation transcriptional responses during periods of limited protein synthesis may therefore depend on translational coupling (an environmentally determined condition) as well as on intrinsic sequence features within protein-encoding genes (a selectable and heritable trait). This could provide bacterial cells a means to specifically govern transcription of certain genes under nonpermissive or weakly permissive conditions for protein synthesis.

RESULTS AND DISCUSSION

Translation through the SECIS element increases transcriptional elongation of *fdhF_{Sec}*. Homoacetogens, like the spirochete *T. primitia*, use formate dehydrogenase enzymes to perform a vital function in the gut microbial communities of termites. These bacteria use the acetyl coenzyme A (acetyl-CoA) pathway for lithotrophic metabolism via the reduction of CO_2 with H_2 , producing acetate as the end product of this anaerobic respiration (18–21). In some termites, acetate generated through the acetyl-CoA pathway meets ca. one-fifth to one-third of the insect's energy demand (22). In return, homoacetogenic bacteria enjoy near-saturating levels of hydrogen in the termite gut, which fuels their metabolism (23). *T. primitia* possesses two paralogous isozymes of formate dehydrogenase (encoded by *fdhF_{Sec}* and *fdhF_{Cys}*). *fdhF_{Sec}* encodes an enzyme that uses a catalytic selenocysteine, whereas *fdhF_{Cys}* encodes a selenium-independent enzyme that instead uses a catalytic cysteine, presumably for periods of selenium scarcity (11). Homologs of both isozymes are pervasive in the gut communities of a wide variety of termite species, suggesting a role for selenium dynamics in shaping the evolution of termite gut homoacetogens (11, 24–26).

We previously investigated the influence of selenium on the transcription of *fdhF_{Cys}* and *fdhF_{Sec}* in *T. primitia* (11). Those studies showed that selenium limitation increases overall transcript levels of *fdhF_{Cys}* but decreases transcript levels of *fdhF_{Sec}* only near the 3' end of the gene. The results are consistent with *fdhF_{Sec}* transcription being initiated but prematurely terminated or paused under growth conditions in which selenium is scarce. Here we used quantitative reverse transcription-PCR (qRT-PCR) to map the locations in *fdhF_{Sec}* where premature termination/pausing may occur. Seven forward and reverse primer pairs were designed to generate a "sliding window" of ca. 100-bp amplicons (Fig. 1A) to measure transcript levels over a range of nucleotide positions near the beginning of *fdhF_{Sec}* in *T. primitia* cultures amended with selenium and in control cultures that were not amended with selenium. To eliminate amplification bias among the different primer sets, the signal of each amplicon in control cultures was divided by the signal from the corresponding amplicon in selenium-amended cultures. A plot of these fractions as a function of nucleotide position produces a curve in which ordinate values of <1 indicate that the level of transcript is higher in selenium-amended cultures than in control cultures (Fig. 1B). These results show that *fdhF_{Sec}* mRNA is prematurely terminated/paused with greater frequency in control cultures than in

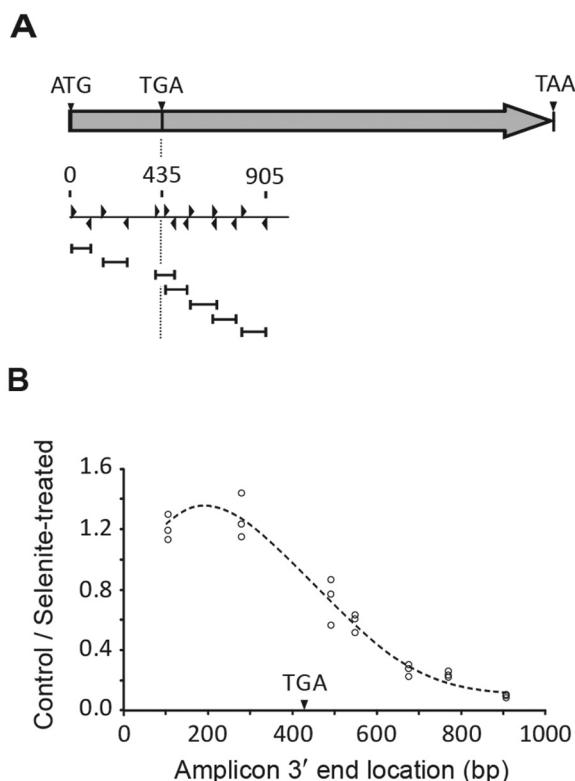


FIG 1 Influence of selenium on transcription of *fdhF_{Sec}* over gene position and time. (A) Location of qRT-PCR primers used for measuring transcript levels. “TGA” marks the position of the selenocysteine codon (amino acid 145). The SECIS element for selenocysteine incorporation occupies nucleotide positions 3 to 46 bp downstream from this codon. Locations of amplicons generated by primers used for mapping transcriptional termination/pausing are indicated by horizontal bars. (B) Influence of selenium (50 nM sodium selenate) on transcript levels over a range of nucleotide positions in *fdhF_{Sec}*. Symbols indicate the relative signal intensities of measurements from controls divided by those of treated cultures and correspond to the 3′ positions of amplicons indicated in panel A. The individual results of three biological replicates are plotted; each data point is the average of duplicate qRT-PCR measurements. The dashed line is the trend line through the approximate average of these measurements. The third and fourth measurements (located between 400 and 600 bp downstream from the start of *fdhF_{Sec}*) include portions of the SECIS element.

selenium-treated cultures over a range of nucleotide positions between 300 and 700 bp downstream from the start of the open reading frame. The inflection point of the curve centers on amplicons that include portions of the SECIS element located from 438 to 481 bp downstream from the start of *fdhF_{Sec}*.

A mechanism by which a deficit of selenium uncouples translation from transcription in *fdhF_{Sec}* can account for our results. In the absence of selenium (and thus selenocysteine), translating ribosomes would likely stall or terminate translation at the selenocysteine codon (UGA), thus uncoupling translation from transcription. The stem-loop mRNA secondary structure formed by the SECIS element (see Fig. S3 in the supplemental material) might then act as a barrier to transcription (analogous to stem-loop structures involved in attenuation of, for example, the *trp* operon), causing premature termination/pausing of the *fdhF_{Sec}* transcript. The range of nucleotide positions over which termination occurs could reflect imprecise termination at the SECIS element combined with the process of 3′-to-5′ mRNA degradation.

Our results contrast previous studies performed with the selenocysteine-encoding *fdhF* gene in *E. coli* in which selenium limitation was not observed to influence transcription downstream from the selenocysteine codon (9, 27), a result that we confirmed in *E. coli* using our own quantitative RT-PCR assays. One possible explanation for the differences between the two species is that the SECIS element in *E. coli* may not effectively inhibit transcription. Indeed, the SECIS element of *fdhF* in *E. coli* has a different structure than the SECIS element of *fdhF_{Sec}* in *T. primitia* (11). Subtle mutations in the *E. coli* SECIS element lead to very different levels of selenocysteine incorporation and overall translation (28). Furthermore, previous research has shown that *E. coli* is able to (though infrequently) misincorporate cysteine at the selenocysteine codon (9), which would allow some translation to occur past the UGA codon even in the absence of selenium.

If coupling and uncoupling of translation controls the level of mRNA produced downstream from the *fdhF_{Sec}* selenocysteine codon in *T. primitia*, then independent factors that inhibit translation should counteract the effect of selenium on transcription. Furthermore, a translational coupling-and-uncoupling mechanism could also poise the transcriptional machinery to respond rapidly to conditions of sudden selenium availability. We have previously observed that transcription of the selenocysteine translation elongation factor SelB is constitutive in *T. primitia* and is not influenced by selenium levels (11), meaning that if selenocystyl-tRNA can be produced, separate *de novo* protein synthesis should not be required to incorporate selenocysteine into nascent polypeptides.

We tested these hypotheses using quantitative RT-PCR to measure transcript levels upstream and downstream from the selenocysteine codon under two different conditions in *T. primitia* cultures. The cultures were grown without selenium and were allowed to enter logarithmic growth phase (approximately 5 days postinoculation). Under these conditions, transcription of *fdhF_{Sec}* downstream from the selenocysteine codon is inhibited. One culture was subsequently amended with selenium, while the other was similarly amended with selenium and concurrently treated with tetracycline (10 $\mu\text{g ml}^{-1}$), a potent inhibitor of protein synthesis in spirochetes (29). At this tetracycline dose, *T. primitia* cells did not divide, yet they remained motile for at least 72 h posttreatment (see Fig. S4 and Movies S1 and S2 in the supplemental material), indicating that the tetracycline treatment did not kill the cells but effectively inhibited protein synthesis long past the time period of the study.

The fraction of transcript produced downstream compared to upstream of the selenocysteine codon demonstrated that the addition of selenium had a dramatic positive effect on transcript levels downstream of the selenocysteine codon (Fig. 2A). After a short lag, the *fdhF_{Sec}* transcript level downstream of the selenocysteine codon in the culture treated only with selenium increased to an approximately half-maximal level 15 min posttreatment and reached a near-maximal level after 30 min. This transcriptional response can be considered rapid compared to growth rate (30), as the generation time of *T. primitia* cultures grown under these conditions is more than 24 h (see Fig. S4 in the supplemental material). In contrast, there was no increase in downstream *fdhF_{Sec}* transcript levels in the culture that was treated with selenium and tetracycline concurrently. These results show that while selenium availability rapidly increases transcriptional productivity, protein synthesis is ultimately required for downstream transcrip-

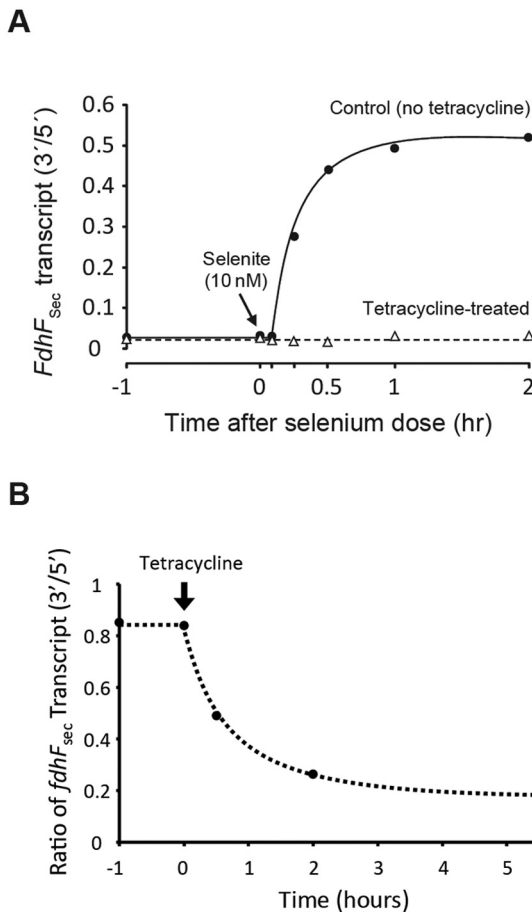


FIG 2 General translation decoupling influences transcription of $fdhF_{Sec}$ in a manner analogous to that of selenium limitation. Levels of downstream and upstream transcript in $fdhF_{Sec}$ were measured by qRT-PCR. (A) Influence of selenium on the transcriptional level upstream and downstream from the selenocysteine codon (and SECIS element) as a function of time. Relative signal intensities are reported as the fraction of signal downstream from the selenocysteine codon divided by the signal upstream. Cultures were initially grown without selenium. The arrow marks the time at which selenium was provided to both cultures. At the same time point, tetracycline was administered to the treatment culture but not the control. (B) The *T. primitia* culture was grown in the presence of selenium (50 nM sodium selenite) for approximately 4 days prior to tetracycline treatment. Unlike conditions under which selenium is not initially added to *T. primitia* cultures, this growth condition promotes high levels of transcription downstream from the selenocysteine codon. At time zero, tetracycline ($10 \mu\text{g ml}^{-1}$) was administered to stop translation.

tion of $fdhF_{Sec}$ in *T. primitia*, a result that is consistent with the conclusions of Proshkin et al. regarding the general requirement of translation for transcriptional elongation (6).

We interpret our results to mean that a selenium deficit in *T. primitia* cells prevents downstream transcription of $fdhF_{Sec}$ by uncoupling translation from transcription at the selenocysteine codon, a condition that is similarly achieved by stopping translation altogether. However, tetracycline did not markedly influence the level of transcript produced upstream from the selenocysteine codon. This was unexpected because, whereas a lack of selenium would stop translation specifically at the selenocysteine codon, tetracycline should prevent translation at or near the beginning of the open reading frame. These results also suggest that selenium is not responsible for inactivating potential RNases involved in de-

grading the 3' end of the transcript when selenium is not provided. Raw qRT-PCR data (see Fig. S5 in the supplemental material) reveal that absolute levels of 5' transcription do not change in cultures treated with selenium alone or concurrently treated with selenium and tetracycline. Therefore, the rise in the fraction of full-length $fdhF_{Sec}$ transcript is due to production of mRNA downstream from the selenocysteine codon. We observe no decrease in low levels of 3' transcript in cultures cotreated with selenium and tetracycline. This probably reflects the fact that a minimal but steady amount of 3' $fdhF_{Sec}$ mRNA is being produced by *T. primitia* under the conditions tested.

One possibility that could account for the selenium-induced increase in 3'-to-5' transcript levels in Fig. 2A is that tetracycline may have prevented *T. primitia* from translating a protein factor necessary for a transcriptional response in $fdhF_{Sec}$ to selenium. To test this, a complementary experiment was performed wherein tetracycline was added to *T. primitia* cells grown under conditions that promote a high level of 3' $fdhF_{Sec}$ transcription (i.e., selenium-replete conditions) and suddenly forced into translation-limiting conditions by the addition of tetracycline (Fig. 2B). This allowed us to test whether or not a lack of translation directly arrests transcription of $fdhF_{Sec}$. We found that adding tetracycline decreased the fraction of 3' $fdhF_{Sec}$ transcripts even though selenium levels remained unchanged; however, the 3' fraction never reached the low level initially observed in Fig. 2A, over the 6-h time period measured. As the abundance of full-length transcripts depends on both production and degradation of RNA, this decrease in 3' transcript levels is likely due to the role of RNases in 3' mRNA degradation of older $fdhF_{Sec}$ transcripts that were transcribed under permissive conditions, while new transcripts, which we presume are not transcribed beyond the SECIS element, are no longer able to compete with RNase degradation beyond this transcriptional elongation stopping point.

So do selenium limitation and exposure to tetracycline affect transcription in comparable ways? To address this question, we performed high-throughput RNA sequencing (RNA-Seq) of *T. primitia* cells during log-phase growth in three cultures initially grown without selenium; one culture was subsequently treated with selenium alone, one was treated with selenium plus tetracycline, and a control culture received neither treatment. RNA was extracted and processed from these cultures 6 h posttreatment, a time point selected to allow sufficient time for transcriptional differences between antibiotic-treated and nontreated cultures to accumulate (Fig. 2B). RNA-Seq was performed in short-read mode (37 bp, nonpaired reads), allowing read depth to be used as a measure of transcript level (31). This approach was used to evaluate the relationship between translation and transcription in $fdhF_{Sec}$ by precisely mapping the location of transcriptional termination under each of these conditions.

All samples produced comparable RNA-Seq data set libraries (Table 1; also, see Materials and Methods). To limit the number of perturbations due to sample handling, no attempt was made to remove rRNA. We normalized genome-wide read depth in selenium-treated and selenium- and tetracycline-treated samples to that of the control sample based on small (ca. 10%) differences in the read depth for rRNA transcripts; however, our results were not markedly influenced by this normalization. To enhance detection of features along the genome, data were averaged over a moving average of 200-bp regions using the MATLAB computational software package. While RNA-Seq data show that read depth dra-

TABLE 1 Overview of RNA-Seq output

Culture	No. of:		
	Total sequences	Mapped sequences	Sequences mapping to non-16S or 23S genes
Selenium treated	13,234,333	11,785,615	413,300
Selenium + tetracycline treated	12,158,875	10,990,439	489,708
Control	13,623,479	11,878,141	511,495

matically decreases over a similar range of nucleotide positions in *fdhF_{Sec}* in all three cultures, the culture treated with selenium alone has a much greater read depth downstream of the selenocysteine codon than either the selenium- and tetracycline-treated or the untreated control culture (Fig. 3). This result is consistent with qRT-PCR data. Apart from a slightly elevated read-depth upstream from the selenocysteine codon in the selenium- and tetracycline-treated sample, the transcriptional pattern resulting from treating cells concurrently with tetracycline and selenium was similar to the nontreated control. Thus, inhibiting protein synthesis altogether in *T. primitia* cells indeed affects transcription downstream from the selenocysteine codon in *fdhF_{Sec}* in a way that is equivalent to the effect of selenium limitation.

The most simple and parsimonious explanation for these results involves a combination of translational uncoupling and transcriptional inhibition by mRNA secondary structure. Our results support the conclusion that uncoupling translation from transcription alone is not sufficient to inhibit transcription, as the 5' transcript level of *fdhF_{Sec}* remains high in the face of translational uncoupling. Instead, transcriptional truncation is likely the effect

of this uncoupling (either generally via the effect of tetracycline or specifically via selenium limitation) combined with RNAP encountering a particular mRNA secondary structure that prematurely terminates or pauses transcription.

While many other mRNA structures are predicted to form in *fdhF_{Sec}* (32), the conspicuous location of mRNA truncation suggests that the SECIS element may act as a potent terminator of transcription under all of the conditions tested (Fig. 3). In the case of cultures treated with selenium alone, some premature transcriptional termination or pausing near the SECIS element could be due to inefficient translation of the selenocysteine codon under our growth conditions. However, so long as translation is coupled to transcription, RNAP can proceed with higher throughput and thus synthesize greater amounts of full-length *fdhF_{Sec}* mRNA. These results show that the trace element selenium can control transcription through a translational coupling mechanism.

Far from being a phenomenon found in an auxiliary bacterial gene, this mechanism occurs in an enzyme that is essential to the central metabolism of *T. primitia* and important for the mutualistic relationship it shares with its host. As such, a transcription/translation coupling mechanism that responds rapidly to selenium availability could be highly advantageous in responding to environmental selenium dynamics.

Selenium availability effects transcription of many *T. primitia* genes. We extended our observations on the effects of selenium to genome-wide transcriptional changes that occur in *T. primitia* by mapping RNA-Seq reads to the closed genome (NCBI reference sequence NC_015578.1). As we intentionally did not enrich for mRNA in this study, our approach reduces the number of genes that can be analyzed because the vast majority (approximately 95%) of RNA-Seq reads map to rRNAs (Table 1). Even with this limitation, these data allow us to reconstruct general effects of limiting selenium availability on transcriptional patterns in *T. primitia* cells (Table 2; also, see Table S2 in the supplemental material).

Selenium availability has a large influence on transcription in *T. primitia*. At least 16 genes are upregulated in response to ele-

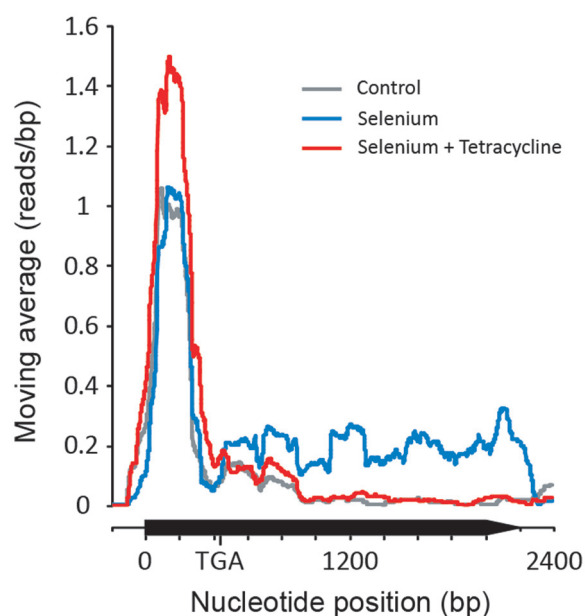


FIG 3 Transcriptional map of *fdhF_{Sec}* by RNA-Seq. Three cultures were initially grown without selenium. One culture was subsequently amended with selenium alone (50 nM sodium selenite) 6 h prior to sampling (blue line). Another culture was subsequently amended with selenium and concurrently with tetracycline 6 h prior to sampling (red line). The control culture received neither treatment (gray line). The lines trace the moving average of Illumina RNA sequencing read depth over a 200 bp sliding-window of nucleotide positions. The black arrow on the x axis indicates the *fdhF_{Sec}* open reading frame. The approximate selenocysteine codon location is indicated (TGA).

TABLE 2 Genome-wide transcriptional changes in *T. primitia*^a

Treatment	No. of genes	
	Upregulated ^b	Downregulated ^c
Selenium	16	98
Selenium plus tetracycline	228	453

^a Out of a total of 3,838 predicted protein-encoding genes, 1,495 genes that met a cutoff of ≥ 50 reads per kb for at least one of the conditions were analyzed.

^b Number of genes for which there is a ≥ 2 -fold-higher transcript level compared to the control.

^c Number of genes for which there is a ≥ 2 -fold-lower transcript level compared to the control.

vated selenium levels. Upregulated genes include *fdh_{Sec}*, encoding the selenium-dependent copy of formate dehydrogenase, and several genes encoding components of hydrogenase enzymes that putatively supply formate dehydrogenase with electrons derived from hydrogen to carry out the reduction of CO₂ (33). The overall pattern of upregulated genes is consistent with *T. primitia* cells responding to selenium by increasing their rate of acetogenesis and growth enabled by the production of the selenocysteine form of formate dehydrogenase, which is predicted to have a higher catalytic rate than the selenium-independent form of the enzyme (13). In addition, over 100 downregulated genes were distributed throughout the genome of *T. primitia* under high-selenium conditions (see Table S2 and Fig. S2A in the supplemental material). Viewed another way, these genes are upregulated upon removal of selenium in cultures of *T. primitia* cells growing under selenium-replete conditions.

The large number of metabolic genes preferentially expressed under low-selenium conditions was expected. As formate dehydrogenase is critical to the central metabolic pathway of *T. primitia*, we interpret these results to mean that numerous genes are needed to compensate for a reduced capacity for acetogenic metabolism due to a deficiency in the preferred *fdhF* isoenzyme. Consistent with this hypothesis was the finding that, in addition to numerous hypothetical proteins of unknown function, genes related to motility, stress response, and acquisition of alternative nutrient sources were found to be transcribed at lower levels when the availability of selenium is high (see Table S2 in the supplemental material). These results suggest that life is more challenging for *T. primitia* without selenium than when selenium is available.

Translational inhibition has a broad and varied effect on transcription. We observed an unexpectedly wide variety of transcriptional responses to globally inhibiting translation. Many genes are transcribed at lower levels in tetracycline treated cells (Table 2), an expected result based on the documented influence of translation on transcription. As with *fdhF_{Sec}*, we observed many genes for which tetracycline-mediated translation inhibition causes premature truncation of transcription (for example, see Fig. 4A to C). However, these genes are often part of multigene operons and are located far downstream from putative promoters. The majority of genes we analyzed are not markedly influenced (<50% increase or decrease in transcript levels) by a lack of translation (Table 2; also see, for example, Fig. 4D to F). We also documented numerous genes for which inhibition of translation increases transcriptional levels over the length of the genes (Table 2; also see, for example, Fig. 4G to I). These observations are not limited to individual genes but extend to genes in putative *T. primitia* operons where similar trends occur over several thousand base pairs (Fig. S1). The list of genes that, on average, demonstrate an increase or decrease in expression in response to tetracycline is quite large and is presented in Table S3 in the supplemental material. The genes that are up- or downregulated are distributed throughout the genome and are not localized to any easily defined hot spots on the chromosome of *T. primitia* (see Fig. S2B in the supplemental material).

It appears to be the case in *T. primitia* that translation has a variable effect on transcription. In essence, translation may act as a transcriptional regulatory governor that is capable of decreasing, increasing, or not influencing transcriptional levels of specific genes. Transcriptional responses of many *T. primitia* genes are dependent on translation under conditions of limited protein syn-

thesis, but these effects may also involve mRNA secondary structure determined by the particular nucleotide sequence of the gene. Such relationships between translation and transcription would imply that mRNA secondary structure could help to control the transcription of genes and would allow certain genes to be transcribed even though translation is severely limited or has altogether ceased. Transcriptional effects caused by the presence and location of rare codons in some genes (34), as well as the placement of Shine-Dalgarno-like sequences within an open reading frame (35) and protein factors such as the NUS factors and Rho, may allow additional layers of transcriptional responses to occur during specific translational challenges. We envision a scenario in which several of these factors act together in order to finely regulate the strength and length of transcription. Support for one such cooperative interaction involving mRNA loops with RNA polymerase core enzyme and elongation factors was proposed recently based on crystal structures of an RNAP “paused” transcriptional complex (36). In such complexes, different NUS proteins are thought to either stabilize or destabilize an RNA structure-mediated clamp confirmation that can be paused or processive, and the presence of hairpins is thought to stabilize the duration of the pause (36).

A large subset of *T. primitia* genes are transcribed even when protein synthesis is inhibited. While the transcription of some genes is initiated at slightly higher levels, possibly leading to multiple RNAPs acting in consort and helping to facilitate transcriptional processivity, this is not necessarily the case. Certain genes may instead be preselected for preferential transcription under conditions that severely limit protein synthesis by the intrinsic nature of their nucleotide sequences. Perhaps it is ultimately a lack of a particular secondary structure that allows some genes to be highly transcribed during periods of inhibited protein synthesis. Prior literature on attenuation-regulated genes espouses the view that a specific subset of genes is selected to be preferentially transcribed under conditions where translation is inhibited (1). It is possible that the scope of gene transcription influenced by translation is even larger than envisioned and that the regulation is ordered in strength from genes that are the most highly upregulated through those that are mildly upregulated to those that are not influenced by translation or are even repressed by it. Genes that are upregulated in the absence of translation in *T. primitia* (Table S3) include many translation-related genes, such as ribosomal machinery, amino acid uptake, and classical attenuation-prone tRNA and amino acid synthesis genes. In addition to these targets, the transcription of several stress response genes is also upregulated under translation-limiting conditions.

While mRNA degradation certainly plays a role in many of the transcriptional patterns observed using qRT-PCR and RNA-Seq approaches, we believe that changes in transcriptional processivity account for the genome-wide changes in transcriptional patterns that we observed, because numerous genes (and putative operons) were not influenced by inhibiting translation. It could also be the case that mRNA secondary structure serves to inhibit 3' RNA degradation at certain locations within genes, but we believe that such a phenomenon alone does not account for our results and would provide no particular advantage to the cells.

Whatever the exact mechanisms that are responsible for these responses may be, we hypothesize that the various links between translation and transcription allow evolutionary processes to act upon certain genes such that transcription is constant or even

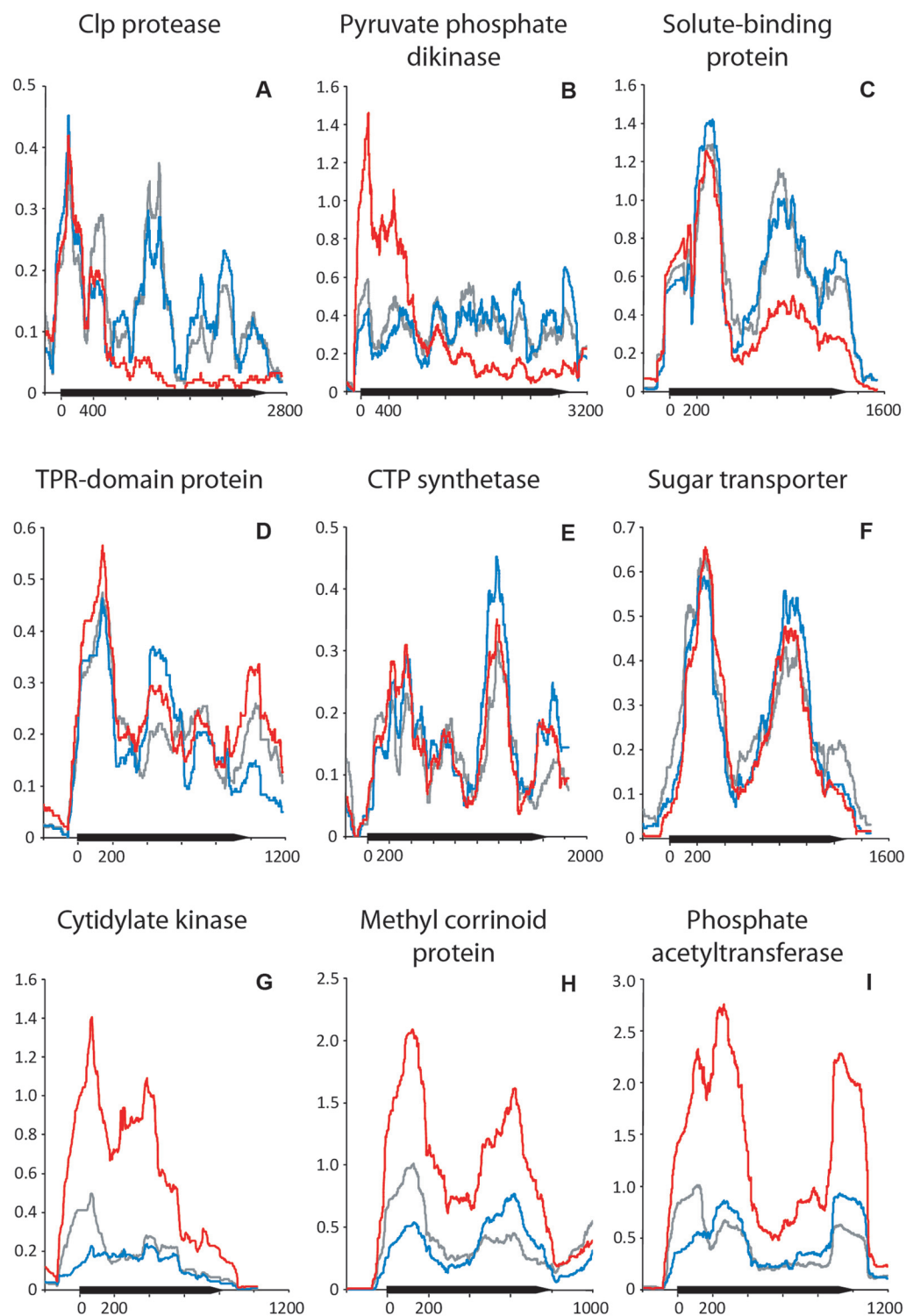


FIG 4 RNA-Seq transcriptional maps of *T. primitia* genes show a variety of transcriptional responses to translation inhibition. The cultures analyzed were the same as in Fig. 3. The three cultures were initially grown without selenium and were subsequently amended with selenium alone (50 nM sodium selenite) 6 h prior to sampling (blue line) or selenium and tetracycline concurrently 6 h prior to sampling (red line). The control culture (gray line) received neither treatment. Traces in each panel show the moving average of Illumina RNA sequencing read depth over a 200-bp sliding window of nucleotide positions. The black arrow on the x axis of each panel indicates the open reading frame of the gene. (A to C) Examples of genes for which transcription is truncated in the culture treated with tetracycline compared to other conditions. Putative gene products are ATP-dependent Clp protease (ATP-binding subunit, ClpA) (A), pyruvate phosphate dikinase (B), and extracellular solute-binding protein (C). (D to F) Examples of genes for which transcription is similar in the culture treated with tetracycline to other conditions. Putative identities are TPR (tetratricopeptide repeat) domain protein (D), CTP synthetase (E), and multiple sugar ABC transporter (substrate-binding subunit) (F). (G to I) Examples of genes for which transcript levels are higher over the length of the gene in the culture treated with tetracycline than under other conditions. Putative identities are cytidylate kinase (G), methyl corrinoid protein (H), and phosphate acetyltransferase (I).

increased during periods of limited protein synthesis. Most bacterial cells in natural environments are not in a state of sustained logarithmic growth but undergo a perpetual cycle of starvation interrupted by periods of nutrient influxes that allow sporadic growth (37). Genes for which transcription is not dependent on translation could be transcribed and thus translated at low levels even under the most stringent of conditions for protein synthesis and could be poised for immediate translation once conditions again become permissive.

Our studies were performed in an organism that belongs to the deeply branching phylum of bacteria known as *Spirochaetes*. Many spirochetes are important pathogens of humans and other animals; however, with the exception of a few examples, such as *Borrelia burgdorferi* (38), most, including *T. primitia*, are currently genetically intractable, meaning that mutational analysis is currently not possible. Our results warrant additional studies into the complexity of post-initiation transcriptional responses, especially in model organisms, such as *E. coli*, where genetic manipulation can easily be performed. Additionally, a new *in vitro* coupled transcription-translation assay which uses the translation components of *E. coli* has been developed for precisely such purposes (39). Such investigations may reveal a greater breadth of functional genes preferentially transcribed under conditions of inhibited protein synthesis as well as the nature of mRNA secondary structure or other, as-yet-unknown factors that may be responsible for prematurely terminating or pausing transcription of genes during inhibited protein synthesis.

MATERIALS AND METHODS

Bacterial cultivation. To support rapid growth, *Treponema primitia* strain ZAS-2 cells were routinely grown in anaerobic YACo broth cultures containing 4% yeast autolysate under an atmosphere of 80% H₂ and 20% CO₂ as previously described (10) and were amended with 20 mM maltose. For experimental samples, cultures were not amended with maltose in order to promote growth on H₂ and CO₂ via the acetyl-CoA pathway. All cultures used in these studies were passaged a minimum of three times under conditions specifically designed to limit the amount of selenium present, as previously described in detail (11). When selenium was added to experimental cultures, it was added in the form of sodium selenite (Sigma, St. Louis, MO), yielding a final concentration of 50 nM, which was previously shown to maximize downstream transcription of *fdhF_{Sec}* (11). Where tetracycline was used to inhibit translation, it was added as tetracycline HCl (Sigma) at a final concentration of 10 μ g ml⁻¹.

Primers. Sequences and related information for primers used in this study are listed in Table S1 in the supplemental material.

RNA extraction and reverse transcription. Upon harvesting of cells (5 ml per sample) from 50-ml total volumes of *T. primitia* cultures grown anaerobically in 300-ml sidearm flasks, RNA was immediately stabilized by the addition of 10 ml of RNeasy lysis reagent (Qiagen, Valencia, CA). Cells were pelleted via centrifugation (5,000 \times g for 10 min), and total RNA was extracted using RNeasy minicolumns with on-column DNase I treatment (Qiagen). RNA was then subjected to a second, 30-min, 37°C off-column DNA digestion using RQ1 DNase enzyme (0.1 U μ l⁻¹) in 1 \times DNase buffer (Promega Corp., Madison, WI). Following the second digest, RNA samples were again purified with RNeasy columns to remove the DNase enzyme.

For quantitative RT-PCR, RNA samples (500 ng RNA in total) were immediately converted to cDNA by randomly primed reverse transcription using iScript reverse transcriptase and cDNA synthesis premix (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's protocol. Duplicate samples lacking reverse transcriptase were prepared for each RNA sample as a negative control to assess residual DNA contamination. In all samples, DNA contamination was below the cycle threshold

(C_T) after 34 of 44 cycles of quantitative RT-PCR, far below the C_T of all mRNA measurements.

For RNA-Seq, samples were prepared in the manner previously reported (40). Briefly, total RNA (at least 5 μ g) was fragmented using an Ambion RNA fragmentation kit (Life Technologies, Grand Island, NY) and then converted to single-strand cDNA using an Invitrogen SuperScript II kit (Invitrogen, Carlsbad, CA). Second-strand buffer (500 mM Tris-HCl [pH 7.8], 50 mM MgCl₂, 10 mM dithiothreitol [DTT]), deoxynucleoside triphosphate (dNTP) (0.3 mM), RNase H (2 U μ l⁻¹; Invitrogen) and DNA polymerase I (Invitrogen) were then added to the first-strand reaction to synthesize second-strand cDNA (16°C, 2.5 h).

Quantitative RT-PCR. Primers for qRT-PCR were designed using Primer3 release 1.0.1 (41) to amplify regions of the *T. primitia* selenocysteine-encoding formate dehydrogenase gene (*fdhF_{Sec}*) upstream and downstream of the selenocysteine codon. Primer sequences are provided in Table S1 in the supplemental material. qPCR was performed in 15- μ l reaction volumes of iQ SYBR green supermix (Bio-Rad Laboratories) using 25 ng of cDNA per reaction and forward and reverse primers (10 pmol each) in separate reactions. A parallel set of reactions was performed for each primer set using a 10-fold dilution series of *T. primitia* genomic DNA as the template to generate standard curves. Transcript levels for the *T. primitia* gene *clpX* were used as an endogenous qRT-PCR control to normalize for sample handling in all PCRs except for samples from cultures treated with tetracycline. This gene was previously shown to be constitutively transcribed and not influenced by selenium (11). The primers QclpXF and QclpXR described in that study were used here. Thermocycling and amplification detection were performed using a Bio-Rad DNAEngine thermocycler outfitted with a Chromo4 real-time detector. Thermocycling conditions for all quantitative PCRs were initial denaturation at 95°C for 3 min followed by 44 cycles of 95°C for 15 s and 60°C for 30 s.

RNA sequencing and data analysis. cDNA samples were submitted to the Caltech Sequencing Core facility (Pasadena, CA). Libraries were sequenced as 37-mers using the standard Illumina protocol and pipeline. Sequencing depth information is summarized in Table 1. Illumina data were aligned to a FASTA file of the *T. primitia* ZAS-2 genome using GERALD (a software package within the Illumina pipeline) and the Maq short-read aligning program (Wellcome Trust Sanger Institute, Hinxton, United Kingdom). Gene expression values were determined by normalizing the number of reads mapped to a particular gene divided by the size of the gene. The resulting value is the normalized reads per kilobase, consistent with the gene expression index calculations of previously published reports (40, 42). In order to adjust for intensity between samples, the ribosomal signal from each sample was used as a standard, and each sample's intensity was multiplied by a factor that would yield an equal rRNA signal. In considering up- or downregulated genes, a cutoff of a 2-fold increase in transcription intensity was used. Additionally, only genes with more than 50 adjusted hits per kb of coding DNA were considered in analyses. Signal intensities were visualized graphically by converting Maq-aligned reads into a BAR file using Cisgenome software and viewed on the Cisgenome browser (Stanford University, Stanford, CA) (43). For a smoother graphical display of data, a moving average of reads per 200-bp sliding nucleotide window was generated using the MATLAB software package (R2011), and corresponding data were again visualized on the genome browser. Genomic circular representation diagrams were generated using the DNAPlotter program (44). All raw data have been deposited in the datadryad website (<http://datadryad.org>).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00869-13/-/DCSupplemental>.

Movie S1, AVI file, 8.6 MB.

Movie S2, AVI file, 8 MB.

Figure S1, PDF file, 0.2 MB.

Figure S2, PDF file, 0.1 MB.

Figure S3, PDF file, 0.1 MB.

Figure S4, PDF file, 0 MB.
 Figure S5, PDF file, 0.1 MB.
 Table S1, PDF file, 0.1 MB.
 Table S2, PDF file, 0.1 MB.
 Table S3, PDF file, 0.1 MB.

ACKNOWLEDGMENTS

This work was supported by the DOE (DE-FG02-07ER64484), and the Center for Environmental Microbial Interactions (CEMI) at Caltech.

We thank Igor Antoscheckhin and the Jacobs Genetics and Genomics Laboratory at Caltech for help with next-generation sequencing and Fabien Paulot for his help with RNA-Seq data analysis.

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